

Biological Analysis Report

SO-DIST-2018-12-18-02

Florida Department of Environmental Protection
Central Laboratory
2600 Blair Stone Road
Tallahassee, FL 32399-2400
DOH Accreditation E31780

Overflow Analyses Performed By:
Benchmark EnviroAnalytical, Inc. - North Port
1001 Corporate Avenue, Suite 102
North Port, FL 34289
DOH Accreditation E85086

Event Description: **Hawthorne & Hatchett**
Request ID: **RQ-2018-12-17-41**
Customer: **SO-DIST**
Project ID: **SMP**

Send Reports to:
FL Dept. of Environmental Protection
FLDEP South District Office
2295 Victoria Ave. Suite 364
Fort Myers, FL 33901-3381
Attn: Maryann Dugan

For additional information please contact
Cheryl Swanson - Administrator
Jacqueline Savage - Microbiology
Diane Power - Bench Biology
Andrew Tintle - Aquatic Toxicology
Puja Jasrotia, Ph.D. - Molecular Biology
Amelia Rankin - Taxonomy (Invertebrate & Algal)
Thekkekalathil Chandrasekhar, Ph.D, QA Officer
Phone (850) 245-8177

Certified by: Cheryl A. Swanson, Program Administrator

Date Certified: 29-JAN-2019 15:59



Case Narrative

Unless otherwise noted, all samples included in this report were received in accordance with protocols referenced in Chapter 62-160, Florida Administrative Code (F.A.C.). Results published in this report pertain only to the samples as submitted to, and received by the laboratory. All times in this report are adjusted to the applicable Eastern Time Zone (EST or EDT).

Results for the following analytical groups are included in this report: Molecular and Overflow.

Scientific notation may be used in reporting very large or small values. Values reported using scientific notation will take the form of the following example: 1.3E+03, which is equivalent to 1.3×10^3 or 1300.

Unless otherwise noted, analytical values for soil and sediment samples are reported on a dry weight basis, and analytical values for waste and tissue samples are reported on a wet weight basis.

Results for TNI accredited tests met requirements established by The NELAC Institute. A double asterisk (**) is used to indicate an analyte/matrix/method for which the laboratory is not TNI accredited or where accreditation for that field of testing is not applicable.

Any significant anomalies or deviations from established protocols are documented in Non-Conformance Reports, which, where appropriate, are included within this analytical report. Additional comments related to specific analytical tests may be included as remarks following the analytical results for each sample. Such comments and remarks are for informational purposes only and are not intended to convey judgement about the usability of the reported data.

A quality control report on the performance of the test method for the submitted samples is included. Uncertainty associated with the analytical results contained in this report can be estimated from the reported quality assurance results and from published quality control acceptance limits for each analytical test. Matrix quality control results (matrix spike recoveries and matrix sample precision) pertain only to the matrix sample tested and do not necessarily reflect test method performance for other samples.

Typical matrix quality control (QC) measurements may include matrix spike recovery, matrix spike duplicate recovery, matrix spike precision and matrix sample precision. Not all matrix QC results may be available or reportable; where they are not an explanation is provided. Typical reasons for unavailable QC results include, but are not limited to, a) insufficient matrix sample to perform some or all QC measurements; b) analyte concentration in the sample replicated was too low for a meaningful measurement of precision and c) analyte concentration in the matrix sample spiked was too high (relative to the amount of analyte spiked) for a meaningful measurement of recovery. Where matrix QC results are unavailable, other method performance metrics (e.g., LCS recovery, LCS precision, surrogate recovery) may be used to assess performance of the method. Comments explaining any missing QC measurements are not intended to convey any adverse conclusions about the quality of the reported data.

Precision is reported as relative percent difference unless otherwise noted.

Quality Control codes as defined below may be used in this report to indicate results that are associated with one or more quality control elements which did not fall within established test method criteria. Such results may be qualified as estimates using a J qualifier as required by 62-160 F.A.C. Explanations are included in the report for any results that were reported as estimates for other reasons.

QC Codes used in this report may include:

- LCS – Recovery for the batch Laboratory Control Sample (LCS) was outside existing control limits;
- MS – Recovery for the batch matrix spike (MS) was outside existing control limits;
- CCV – Recovery for a continuing calibration verification (CCV) standard was outside existing control limits;
- SUR – Recovery of a surrogate (SUR) for associated analytes was outside existing control limits;
- RPD – The precision, measured as relative percent difference (RPD), of batch replicate measurements was outside existing control limits;
- RSD – The precision, measured as relative standard deviation (RSD), of batch replicate measurements was outside existing control limits;
- SMP – Sample - used precision derived from replicate analyses of a sample;

The following data qualifiers are used, where applicable, in this report as specified in 62-160 F.A.C.

- A - Value reported is the mean of two or more determinations.
- B - Results based on colony counts outside the acceptable range.
- I - The reported value is between the laboratory method detection limit and the laboratory practical quantitation limit.
- J - Estimated value and/or the analysis did not meet established quality control criteria.
- K - Actual value is known to be less than value given.
- L - Actual value is known to be greater than value given.
- N - Presumptive evidence of presence of material.
- O - Sampled, but analysis lost or not performed.
- Q - Sample held beyond normal holding time.
- T - Value reported is less than the criterion of detection.
- U - Material was analyzed for but not detected. The reported value is the method detection limit for the sample analyzed.
- V - Analyte was detected in both sample and method blank.
- X - Too few individuals to calculate SCI value.
- Y - The laboratory analysis was from an unpreserved or improperly preserved sample. The data may not be accurate.
- Z - Colonies were too numerous to count (TNTC).

Quality control information from overflow laboratories may not be included in this report. Please refer to the associated report from the overflow laboratory for additional information.

Sample Location: HawthorneCrk@ReynoldsSt

Collection Date/Time: 12/17/2018 10:55

Field ID: G3SD0011

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2049497	SOP-PCR-1.0	HF183-qPCR**	250	I	GEU/100 mL	P356791	
	SOP-PCR-4.0	HF183 - Human Source Marker**	Above Threshold				
2049499	SM 9223 Quanti-Tray	Escherichia Coli-Quanti-Tray	1201		MPN/100 mL		

Sample Location: HatchettCrk@PinebrookRd

Collection Date/Time: 12/17/2018 12:50

Field ID: G3SD0103

Matrix: W-SURF-FRH

Sample ID	Ref. Method	Component	Result	Code	Units	Batch ID	QC Codes
2049498	SOP-PCR-1.0	HF183-qPCR**	94	U	GEU/100 mL	P356791	
	SOP-PCR-1.2	GULL2-qPCR**	1.1E+04	U	TSC/100 mL	P356791	
	SOP-PCR-1.3	GFD-purified-qPCR**	110	UJ	TSC/100 mL	P356978	MS
	SOP-PCR-1.4	BacR-qPCR**	2.0E+03	U	TSC/100 mL	P356791	
	SOP-PCR-4.0	HF183 - Human Source Marker**	Above Threshold				
2049500	SM 9223 Quanti-Tray	Escherichia Coli-Quanti-Tray	496		MPN/100 mL		

Ref. Method and Comment:

SOP-PCR-1.2: Precision data unavailable for Analysis Batch A89000

SOP-PCR-1.3: Quality control failure(s) observed. See QA report for details.

SOP-PCR-1.4: Precision data unavailable for Analysis Batch A88987

Quality Assurance Report Method Blank Results

Reference Method: SOP-PCR-1.0

Batch ID: P356791

Component	Result	Code	Units
HF183-qPCR	280	U	GEU/100 mL

Reference Method: SOP-PCR-1.2

Batch ID: P356791

Component	Result	Code	Units
GULL2-qPCR	1.7E+04	U	TSC/100 mL

Reference Method: SOP-PCR-1.3

Batch ID: P356978

Component	Result	Code	Units
GFD-purified-qPCR	340	U	TSC/100 mL

Reference Method: SOP-PCR-1.4

Batch ID: P356791

Component	Result	Code	Units
BacR-qPCR	3.0E+03	U	TSC/100 mL

Quality Assurance Report Laboratory Control Sample Accuracy

Quality Assurance Report Laboratory Control Sample Accuracy

Reference Method: SOP-PCR-1.0
Batch ID: P356791

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
HF183-qPCR	114	132	P/P	50 - 175

Reference Method: SOP-PCR-1.2
Batch ID: P356791

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
GULL2-qPCR	144	171	P/P	50 - 300

Reference Method: SOP-PCR-1.3
Batch ID: P356978

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
GFD-purified-qPCR	161	57.5	P/P	50 - 175

Reference Method: SOP-PCR-1.4
Batch ID: P356791

Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
BacR-qPCR	92.6	100	P/P	50 - 175

Quality Assurance Report Matrix Spike Accuracy

Reference Method: SOP-PCR-1.0
Batch ID: P356791

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2049497	HF183-qPCR	110		P	50 - 175
2049498	HF183-qPCR	89.6		P	50 - 175
2049831	HF183-qPCR	90.5		P	50 - 175
2050247	HF183-qPCR	120		P	50 - 175
2050248	HF183-qPCR	107		P	50 - 175
2051109	HF183-qPCR	94.4		P	50 - 175
2051189	HF183-qPCR	87.1		P	50 - 175
2051648	HF183-qPCR	107	116	P/P	50 - 175
2053632	HF183-qPCR	68.3		P	50 - 175
2054264	HF183-qPCR	96.4		P	50 - 175
2054265	HF183-qPCR	119		P	50 - 175

Reference Method: SOP-PCR-1.2
Batch ID: P356791

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2049498	GULL2-qPCR	120		P	50 - 300
2049831	GULL2-qPCR	98.1		P	50 - 300
2050248	GULL2-qPCR	111		P	50 - 300
2051109	GULL2-qPCR	121		P	50 - 300
2053632	GULL2-qPCR	78.4		P	50 - 300

Quality Assurance Report Matrix Spike Accuracy

Reference Method: SOP-PCR-1.3
Batch ID: P356978

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2049498	GFD-purified-qPCR	198		F	50 - 175
2049831	GFD-purified-qPCR	222		F	50 - 175
2050248	GFD-purified-qPCR	189		F	50 - 175
2051109	GFD-purified-qPCR	216		F	50 - 175
2053632	GFD-purified-qPCR	219	197	F/F	50 - 175

Reference Method: SOP-PCR-1.4
Batch ID: P356791

Spiked Sample	Component	% Rec.1	% Rec.2	Pass/Fail	Control Limits
2049498	BacR-qPCR	82.1		P	50 - 175
2049831	BacR-qPCR	63.2		P	50 - 175
2050248	BacR-qPCR	67.6		P	50 - 175
2051109	BacR-qPCR	73.3		P	50 - 175
2053632	BacR-qPCR	69.5		P	50 - 175

Quality Assurance Report Precision

Reference Method: SOP-PCR-1.0
Batch ID: P356791

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2051648	HF183-qPCR	7.92	Spike	P	0 - 25

Reference Method: SOP-PCR-1.3
Batch ID: P356978

Replicated Lab Sample	Component	% RSD/RPD	Sample/Spike/LCS*	Pass/Fail	Control Limits
2053632	GFD-purified-qPCR	10.6	Spike	P	0 - 25

* Sample, spike and/or laboratory control sample precision (LCS) is reported.
Replicate spike precision may be reported when sample results are below quantifiable levels.

Quality Assurance Report Surrogates

Lab Sample ID: 2049497
Field Sample ID: G3SD0011

Reference Method	Surrogate	% Rec.	Pass/Fail	Control Limits
SOP-PCR-1.0	SKETA-qPCR	94.2	P	50 - 300

Lab Sample ID: 2049498
Field Sample ID: G3SD0103

Reference Method	Surrogate	% Rec.	Pass/Fail	Control Limits
SOP-PCR-1.0	SKETA-qPCR	89.9	P	50 - 300
SOP-PCR-1.2	SKETA-qPCR	116	P	50 - 300

Quality Assurance Report Surrogates

Lab Sample ID: 2049498
Field Sample ID: G3SD0103

Reference Method	Surrogate	% Rec.	Pass/Fail	Control Limits
SOP-PCR-1.4	SKETA-qPCR	86.1	P	50 - 300

Quality Assurance Report Summary

Ref. Method	Analyte	LCS % Recovery	MS % Recovery	LCS	Precision SMP	MS
SOP-PCR-1.0	HF183-qPCR	132 114	107 116 110 89.6			7.92
SOP-PCR-1.2	GULL2-qPCR	171 144	120 111 98.1 121			
SOP-PCR-1.3	GFD-purified-qPCR	57.5 161	198 222 189 216			10.6
SOP-PCR-1.4	BacR-qPCR	100 92.6	82.1 67.6 63.2 73.3			

Reference Method Descriptions

Method / DoH Cert #	Description	Associated Samples
SM 9223 Quanti-Tray / E85086	Escherichia coli by Colilert 18 Quanti-Tray method by Benchmark EA South in North Port	2049499, 2049500
SOP-PCR-1.0 / E31780	Detection and quantification of human-specific HF183 Bacteroides genetic marker with quantitative polymerase chain reaction	2049497, 2049498
SOP-PCR-1.2 / E31780	Detection and quantification of coastal bird specific Catellicoccus marimammalium Gull2 genetic marker with quantitative polymerase chain reaction	2049498
SOP-PCR-1.3 / E31780	Detection and quantification of bird specific Helicobacter GFD genetic marker with quantitative polymerase chain reaction	2049498
SOP-PCR-1.4 / E31780	Detection and quantification of Ruminant specific Bacteroidetes BacR genetic marker with quantitative polymerase chain reaction	2049498
SOP-PCR-4.0 / E31780	Preparation of Samples by Filtration and held for qPCR Analysis	2049497, 2049498

Preparation and Analysis Log

Ref. Method	Received Date	Prep Date/Time	Prepared By	Analysis Date/Time	Analyzed By	Associated Samples
SM 9223 Quanti-Tray	12/17/2018	12/17/2018 15:29		12/18/2018 15:47	Benchmark EnviroAnalytical in North Port	2049499, 2049500
SOP-PCR-1.0	12/18/2018	12/18/2018 12:45	Angela Smith	01/23/2019 10:19	Mailin Lopez	2049497, 2049498
SOP-PCR-1.2	12/18/2018	12/18/2018 12:45	Angela Smith	01/24/2019 12:44	Mailin Lopez	2049498
SOP-PCR-1.3	12/18/2018	12/18/2018 12:45	Sarah Menz	01/28/2019 11:22	Sarah Menz	2049498
SOP-PCR-1.4	12/18/2018	12/18/2018 12:45	Angela Smith	01/24/2019 08:39	Mailin Lopez	2049498
SOP-PCR-4.0	12/18/2018	12/18/2018 12:45	Angela Smith	01/04/2019 10:54	Sarah Menz	2049497, 2049498